COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF A GENE FROM THE EBOLA

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Ser. No. 60/908,793, filed Mar. 29, 2007. The entire contents of this provisional application are hereby incorporated by reference in the present application.

GOVERNMENT SUPPORT

[0002] The work described herein was carried out, at least in part, using funds from the United States government under contract number HHSN266200600012C, ADB N01-AI-60012, from the National Institute of Allergy and Infectious Diseases/National Institutes of Health/Department of Health and Human Services (NIAID/NIH/DHHS). The government may therefore have certain rights in the invention.

FIELD OF THE INVENTION

[0003] This invention relates to double-stranded ribonucleic acid (dsRNA), and its use in mediating RNA interference to inhibit the expression of one of the genes of the Ebola virus and the use of the dsRNA to treat pathological processes mediated by Ebola infection, such as systemic hemorrhage and multi-organ failure.

BACKGROUND OF THE INVENTION

Ebola Virus

[0004] Minus-strand (−) RNA viruses are major causes of human suffering that cause epidemics of serious human illness. In humans the diseases caused by these viruses include influenza (Orthomyxoviridae), mumps, measles, upper and lower respiratory tract disease (Paramyxoviridae), rabies (Rhabdoviridae), hemorrhagic fever (Filoviridae, Bunyaviridae and Arenaviridae), encephalitis (Bunyaviridae) and neurological illness (Bornaviridae). Virtually the entire human population is thought to be infected by many of these viruses.

[0005] The Ebola virus comes from the Filoviridae family, similar to the Marburg virus. It is named after the Ebola River in Zaire, Africa, near where the first outbreak was noted by Dr. Ngoy Mushola in 1976 after a significant outbreaks in both Yambuku, Zaire (now the Democratic Republic of the Congo), and Nzara, in western Sudan. Of 602 identified cases, there were 397 deaths.

[0006] The two strains identified in 1976 were named Ebola-Zaire (EBO-Z) and Ebola-Sudan (EBO-S). The outbreak in Sudan showed a lower fatality rate—50%—compared to the 90% mortality rate of the Zaire strain. In 1990, a second, similar virus was identified in Reston, Va. amongst monkeys imported from the Philippines, and was named Ebola-Reston.

[0007] Further outbreaks have occurred in Zaire/Congo (1995 and 2003), Gabon (1994, 1995 and 1996), and in Uganda (2000). A new subtype was identified from a single human case in the Côte d'Ivoire in 1994, EBO-CI.

[0008] Of around 1500 identified human Ebola infections, two-thirds of the patients have died. The animal (or other) reservoir which sustains the virus between outbreaks has not been identified.

[0009] Among humans, the Ebola virus is transmitted by direct contact with infected body fluids such as blood.

[0010] The incubation period of Ebola hemorrhagic fever varies from two days to four weeks. Symptoms are variable too, but the onset is usually sudden and characterised by high fever, prostration, myalgia, arthralgia, abdominal pains and headache. These symptoms progress to vomiting, diarrhea, oropharyngeal lesions, conjunctivitis, organ damage (notably the kidney and liver) by co-localized necrosis, proteinuria, and bleeding both internal and external, commonly through the gastrointestinal tract. Death or recovery to convalescence occurs within six to ten days of onset of symptomology.

[0011] The development of a successful therapeutic for Ebola virus is a long-sought and seemingly difficult endeavor. Although they cause only a few hundred deaths worldwide each year, filoviruses are considered a significant world health threat and have many of the characteristics commonly associated with biological weapons since they can be grown in large quantities, can be fairly stable, are highly infectious as an aerosol, and are exceptionally deadly. Filoviruses are relatively simple viruses of 19 Kb genomes and consist of seven genes which encode nucleoprotein (NP), glycoprotein (GP), four smaller viral proteins (VP24, VP30, VP35 and VP40), and the RNA-dependent RNA polymerase (L protein) all in a single strand of negative-sensed RNA. Administration of type I interferons, therapeutic vaccines, immune globulins, ribavirin, and other nucleoside analogues have been somewhat successful in rodent Ebola virus models, but not in nonhuman primate infection models.

[0012] In view of the severity of the diseases caused by (−) RNA viruses, in particular members of the Filoviridae family of viruses, and the lack of effective prevention or therapies, it is therefore an object of the present invention to provide therapeutic compounds and methods for treating a host infected with a (−) RNA virus.

[0013] siRNA

[0014] Double-stranded RNA molecules (dsRNA) have been shown to block gene expression in a highly conserved regulatory mechanism known as RNA interference (RNAi). WO 99/32619 (Fire et al.) discloses the use of a dsRNA of at least 25 nucleotides in length to inhibit the expression of genes in *C. elegans*. dsRNA has also been shown to degrade target RNA in other organisms, including plants (see, e.g., WO 99/53050, Waterhouse et al.; and WO 99/61631, Heifetz et al.), *Drosophila* (see, e.g., Yang, D., et al., Curr. Biol. (2000) 10:1191-1200), and mammals (see WO 00/44895, Limmer; and DE 101 00 586.5, Kreutzer et al.). This natural mechanism has now become the focus for the development of a new class of pharmaceutical agents for treating disorders that are caused by the aberrant or unwanted regulation of a gene.

[0015] Recent reports have indicated that in vitro, RNAi may show some promising in reducing Ebola replication and providing protection in guinea pigs (Geisbert, et al., The Journal of Infectious Diseases, 193 (2006), 1650-1657). However, the RNAi agents examined were not designed against all known Ebola strains and were not selected for stability and other properties needed for in vivo therapeutic RNAi agents. Accordingly, despite significant advances in the field of RNAi, there remains a need for an agent that can selectively and efficiently silence a gene in the Ebola virus using the cell's own RNAi machinery that has both high biological activity and in vivo stability, and that can effec-